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Short Communication

Determination of famotidine in plasma, urine and gastric juice by high-performance liquid chromatography using disposable solid-phase extraction columns

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Introduction

Famotidine (FMTD; MK 208; YM-11170) is a recently developed histamine H2-receptor blocker. Its chemical name is 3-[[[2-[(aminoiminomethyl) amino]-4-thiazolyl] methyl] thio]-*N*-(aminosulfonyl)-propanimidamide, its formula is shown in Fig. 1. Because of its high potency, evaluated on a weight basis versus other H2-receptor antagonists, FMTD has been widely studied for the treatment of peptic ulcers.

FMTD is stable for several months in biological samples stored at −15°C [1]. After oral administration, peak plasma concentrations are reached within 1–3 h, with a limited proportion of protein binding (15–22%) [2]. Metabolic studies in rat and dog using ^{14}C FMTD showed that the drug is mainly excreted in urine [3]. In biological fluids FMTD concentrations may vary widely, but the proportion of metabolites (the principal one being the sulphoxide derivative) is relatively small, so that no other relevant peaks of similar molecules may be detected by HPLC.

In order to extend pharmacokinetic studies and to assess patient compliance, a fast and reliable method for the evaluation of the drug in biological fluids is required. Such method [1] for the determination of FMTD in plasma, urine and gastric juice using solid-phase extraction columns is described in the present communication.

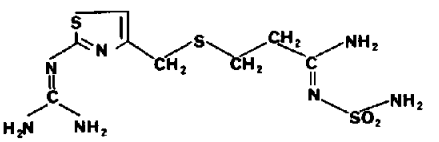

Figure 1
Chemical structure of famotidine.

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Experimental

Chemicals

Famotidine was supplied by Merck, Sharp and Dohme Italia (Rome, Italy). HPLC-grade methanol, *N,N*-dimethylformamide, acetonitrile and all other analytical grade reagents (acetic acid, phosphoric acid) were obtained from Farmitalia-Carlo Erba (Milan, Italy). HPLC-grade water was obtained by double distillation in glass and purification through a Milli-Q water purification system (Millipore, Bedford, MA, USA). Water was filtered through HA 0.45 μm filters, whilst methanol, acetonitrile, and dimethylformamide were filtered through FA 0.5 μm filters (Millipore).

Stock solutions

A 1.0 mg ml^{-1} stock solution of FMTD was prepared in methanol and 100, 10, 1 and 0.1 μg ml^{-1} solutions were sequentially obtained by serial dilution.

Chromatographic system and conditions

The Waters HPLC system (Waters Assoc., Milford, MA, USA) employed consisted of a M6000A pump, a U6K injector and a model 440 fixed-wavelength UV detector (254 nm) connected to a 740 Data Module Integrator.

The extraction apparatus was a Supelco solid phase extraction manifold equipped with a Drying Attachment (Supelco, Bellefonte, PA, USA). Bond-Elut silica columns (3.0 ml capacity) were purchased from Analytichem International (Harbor City, CA, USA).

The separation was performed on an analytical 250 × 4.6 mm i.d. reversed-phase Supelcosyl LC-8 (5 μm, particle size) column protected by a 2 cm disposable Pelliguard column (40 μm; Supelchem, Milan, Italy).

The separations were performed at ambient temperature and the detector set at 0.005 absorbance units full scale. The mobile phase consisted of a mixture of methanol–acetonitrile–0.016 M phosphoric acid (10:10:80, v/v/v), filtered and degassed prior to use. The mobile phase was prepared daily and delivered at a flow rate of 1.0 ml min^{-1}.

Biological samples

Healthy volunteer patients, from whom the informed consent had been obtained, were given a single 40 mg tablet of famotidine. Plasma, urine and gastric juice samples were collected at various times afterwards and extracted prior to HPLC analysis.

Sample preparation

Plasma. Heparinised blood samples from various patients were centrifuged and plasma collected and frozen at −20°C. Samples were thawed just before the extraction procedure, thoroughly agitated and centrifuged at 800 **g** for 30 min. The Bond-Elut cartridges were placed in a luer that fitted the top of the Supelco vacuum manifold, which may be loaded with up to 12 cartridges. A vacuum of 250–500 Torr was applied to the manifold to carry out the various steps of the extraction. A 1 ml rinse of methanol followed by 2 ml of HPLC-grade water served to desorb any organic impurities from the cartridges and to wet the silica packing prior to introduction of plasma samples. Then 1 ml of plasma was run through the cartridge followed by 5 ml of water. The effluent was discarded. Two millilitres of acetonitrile were then applied to the cartridge and the eluate collected. This fraction was finally centrifuged (800 **g** for 15 min), filtered through a Millipore HV (0.45 μm) filter (Millipore, Bedford, MA, USA), evaporated to dryness

with nitrogen stream under vacuum utilising the Supelco Drying Attachment. The samples were then reconstituted to 200 μl, with the mobile phase mixture and mixed on a Vortex agitator. Aliquots of each sample (100 μl) were chromatographed on a Supelcosil LC-8 reversed-phase column at ambient temperature using the described mobile phase. Column eluate was monitored at 254 nm.

Urine. Urine samples were stored frozen (−20°C) until required for assay. Samples were thawed just before the extraction procedure, thoroughly agitated, added with (0.1 ml of methanol and vortexed. After cartridge activation, a sample of 0.5 ml of urine was run through it, followed by 3 ml of water. The effluent was discarded. A 2.0 ml volume of dimethylformamide:water (1:1, v/v) mixture was then applied to the cartridge and the eluate collected. An aliquot of 100 μl of this sample was injected into a chromatograph.

Gastric juice. Samples of gastric juice collected during fiberoptic gastroscopy to patients were frozen. The extraction procedure was identical to that described for plasma samples.

Calibration curves for plasma and urine

Sets of standard plasma and urine samples were prepared by addition of known amounts of FMTD to blank plasma or urine. The chromatographic peak area ratios of FMTD were subjected to linear regression versus the corresponding FMTD concentrations. The resulting equation was used to calculate the concentrations of FMTD in the test samples.

Recovery

Recovery of FMTD from the extraction procedures was determined by comparing the peak area ratios of test samples with blank samples which were spiked with FMTD at the same concentration following extraction.

Results and Discussion

Typical chromatograms of FMTD are illustrated in Fig. 2. They do not contain any interfering peak showing a retention time similar to that of FMTD, which was found to have an average retention time of 9.5 min.

The calibration curve for the determination of FMTD in human plasma was linear over the range 0.1–2.0 μg ml^{-1} and the corresponding regression equation was: $Y = 0.647\ X + 0.006$ ($r = 0.998$), where Y is the peak area ratio to FMTD and X is the FMTD concentration (μg ml^{-1}) in plasma. For gastric juice samples the same parameters of plasma were applied.

For urine the linear range (0.1–5.0 μg ml^{-1}) was described by the equation: $Y = 0.513\ X + 0.045$ ($r = 0.999$), where Y is the peak area for FMTD and X is the FMTD concentration (μg ml^{-1}) in urine.

The detection limits were 0.01 μg ml^{-1} for both plasma and urine, at a signal to noise ratio of 5:1. The precision of the assay was represented by relative standard deviation values of around 4% ($N = 5$) for both, plasma and urine (Table 1). The extraction recovery of FMTD from the human plasma was 93% and did not change between 0.2 and 0.5 ml sample volume. The plasma samples were stable for at least five weeks when

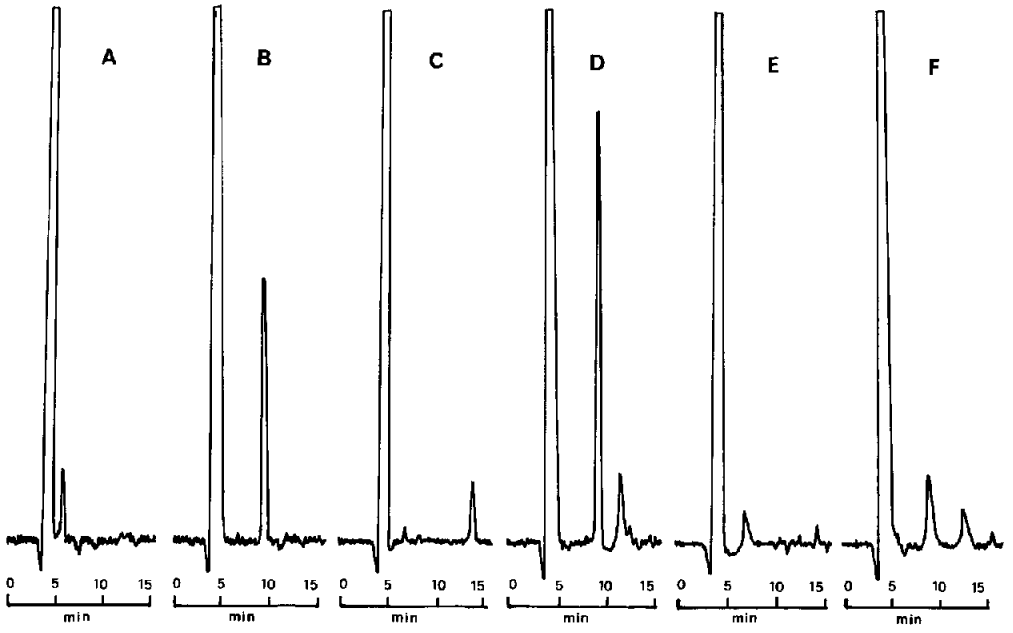

Figure 2
Chromatograms from human plasma, urine and gastric juice extracts: (A) blank plasma; (B) blank plasma spiked with 0.3 μg ml^{-1} FMTD; (C) blank urine; (D) blank urine spiked with 0.5 μg ml^{-1} FMTD; (E) blank gastric juice; (F) blank gastric juice spiked with 0.1 μg ml^{-1} FMTD. Injection volume was 100 μl.

Table 1
Reproducibility of the analysis of FMTD in human plasma and urine

Added amount of famotidine (μg ml^{-1})		Found* Mean ± S.E.	RSD %
Plasma	0.1	0.104 ± 0.002	3.7
	0.3	0.302 ± 0.006	3.5
	0.5	0.505 ± 0.012	2.8
	1.0	1.002 ± 0.005	2.4
	1.5	1.498 ± 0.004	3.2
	2.0	1.997 ± 0.011	2.2
Urine	0.1	0.090 ± 0.007	4.3
	0.5	0.470 ± 0.003	3.5
	1.0	0.990 ± 0.004	4.1
	1.5	1.500 ± 0.058	3.9
	2.0	1.980 ± 0.073	3.7
	5.0	4.980 ± 0.021	2.5

*Mean ± S.E. and relative standard deviation (RSD) of five determinations.

stored at −20°C. Mean recoveries of FMTD from the extraction procedure were as follows: plasma 80 and 87% at 0.5 and 1.0 μg ml^{-1} respectively and urine 85 and 90% at 0.5 and 1.0 μg ml^{-1} respectively.

This simple HPLC method should be of value for monitoring the plasma concentration of FMTD in patients, for assessing patient compliance in assuming prescribed FMTD

regimens and for examining the relationship between FMTD concentration in plasma and anti-secretory effect.

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